PNFI Technical Reports

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POLLINATION TECHNIQUES III. FLOWER DEVELOPMENT RATING AND POLLEN APPLICATION

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Introduction

This is the last in a series of three technical notes on pollination techniques. Information is presented here dealing with field observations for male and female flower development in *Picea mariana, Pinus banksiana,* and *Larix* spp. Female flower isolation techniques and pollen application as conducted at the Petawawa National Forestry Institute (PNFI) are discussed.

Flower Development Rating

Pollen and female flower development classification systems for field use, described in various publications (e.g., Franklin 1981, Villar et al. 1964), are advantageous in determining when to bag female flowers, their receptivity periods, and optimal times for pollen collection and application. An awareness of these systems will give the novice tree breeder a clear picture of what to expect and, therefore, a head start to a successful breeding season.

At PNFI the Tree Genetics and Breeding project has devised a simple rating system to classify female and male flowers based on personal observations of conifer species being studied at the Petawawa Research Forest.

In general, the development of female flowers of *Picea, Pinus,* and *Larix* can be categorized into six stages, beginning in the spring with bud swell and bud break through to the juvenile, intermediate, advanced, and fully receptive stages. Pollen flower development can also be categorized into six stages also, in which the key component is the progression from a juicy extrude to a powder.

These stages are different in each genus and it should be noted that differences exist in flowering features even within a genus, at a species level. Appendices 1-3 show observations for: 1) *Picea mariana,* 2) *Pinus banksiana,* and 3) *Larix* spp.

Isolating female flowers:

Pollination bags are placed over female flowers in the early stages of flower development.

The first step is to strip the branch of needles at the spot where the bag will be tied.

If the branch is weak, a bamboo splint is attached to support the branch.

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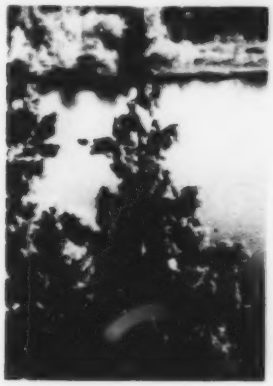
Cotton is wound around the branch (and between the splint).

The pollination bag is then pulled over the branch, making sure that the window is facing the ground and has minimal exposure to the sun.

The bag is then tied securely over the cotton using string.

Often, the work is conducted on tree tops (below), which requires tree climbing skills (see Yeatman and Nieman 1984). Organization of materials is essential and maximum concentration is required for safety reasons and to effectively complete the job.



Applying pollen:

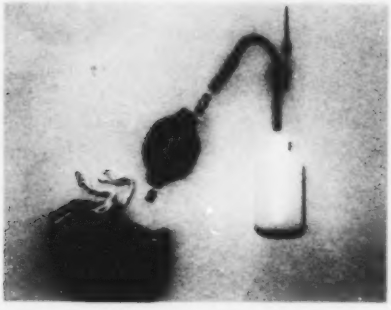
When female flowers are receptive, the pollination bag is pierced with the needle of the pollen applicator. The pollen is then squirted into the bag by squeezing the rubber ball a number of times. Pollen should only then be visible inside the bag.

Immediately after removing the needle from the bag, the hole is sealed with glue to avoid the possibility of airborne pollen contamination. The bag is then shaken to ensure complete dispersal of the pollen.

Equipment used:

The following is a list of equipment and materials used by the Tree Genetics and Breeding Project at PNFI to isolate female flowers and to apply the pollen. They are illustrated in the photograph below.

- POWDER DISPENSER (below) made up of: 120 mL polyethylene bottle, a rubber stopper with two holes, copper tubing, rubber tubing, two one-way valves, a veterinary needle (size 14 to 16), a rubber squeeze ball, a filter sac (cloth bag with drawstrings lined with cotton).



- POLLINATION BAG: Currently used is the standard 8 x 20 inch (20 x 50 cm) bag with a plastic strip along one face. It suits most of our requirements; however, bags of various sizes would be beneficial. A word of caution...if these bags are to be used after some time in storage, check each one before use to ensure the glue securing the folded top is still holding.
- ROLLED SYNTHETIC COTTON. We use a product called 'Beauty Coil' which can be purchased through a supplier to hair dresser shops. Other brands will be just as suitable.
- CONTACT GLUE
- BAMBOO SPLINTS
- STRING or PULL TIES
- plastic horticultural LABELS and permanent MARKER PENS.

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Appendix 1a

A development rating system for *Picea mariana* female flowers (megastrobili)

The bud scales appear to flake off giving an impression of slow flower development. However, when the emerging flower is sighted, the development is well into the intermediate stage (stage 3) and is rapidly advancing.

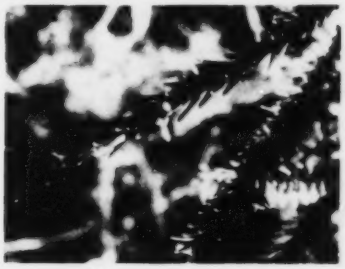
Pollination should be conducted when female flowers are at late stage 5. For security, two pollen applications 1 to 2 days apart should be made during this period of receptivity.

Stage

0 - bud is distinguishably larger than the vegetative buds, and pointed

1 - bud is beginning to enlarge and is glossy in appearance
- bud scales tight

Stage 1



2 'juvenile' stage:
- bud scales loosening up, appearing somewhat flaky
- the purple colour of the flower barely shows

Stage 2

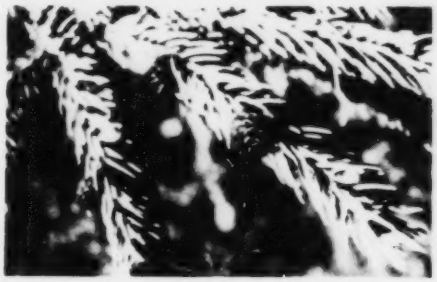
3 'intermediate' stage:
- bud scales flaking away exposing the purple flower
- cone scales beginning to push away bud scales and opening up

Stage 3

4 'advanced' stage:
- cone scales fully exposed and approaching a position at right angles to the cone axis

5 'receptive' stage:
- cone scales spread fully open (more or less at right angles to the cone axis)

Stage 5



Bagging can be done at stages 1 or 2. As the flowers emerge they become fragile and can easily break off if accidently hit. Early bagging will contribute to the integrity of the controlled cross as well as allow one to concentrate on pollen collection. It should be noted, however, that early bagging may promote flower development. Because early collection and forced extraction of pollen requires detailed attention (see Copis 1990 a,b) in black spruce, close observation must be paid to both male and female flower development to achieve synchrony.

Appendix 1b

A development rating system for *Picea mariana* male flowers (microstrobili)

Stage

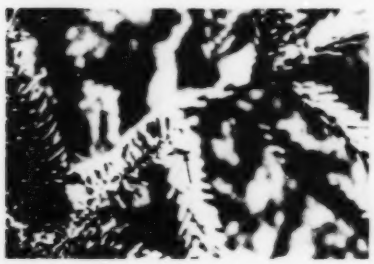
0 - small round buds covered tightly with bud scales (arrow)

1 - bud scales beginning to flake away, barely exposing the purple colour of the pollen flower

Stages 0-1

2 'juvenile' stage:
- bud scales still evident (arrow)
- a clear fluid squirts out of the pollen flower when squeezed

Stage 2



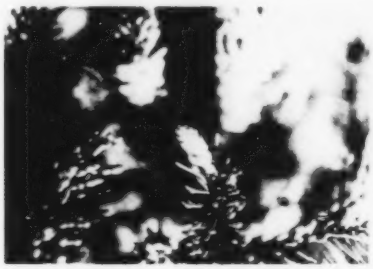
3 'intermediate' stage:
- appearance is more robust than before
- bud scales have receded completely, exposing the pollen flower
- a yellow fluid is emitted when squeezed

Stage 3

4 - 'advanced' stage:
- the pollen flower is swollen and quite limber
- when squeezed the flower does not squirt fluid but rather emits a thick yellow paste which almost dries to a powder when rolled between the fingers

5 'mature' tage:
- the microstrobilus is beginning to "burst"
- when squeezed it feels spongy and no liquid is emitted
- the pollen flower when flicked with one's finger will freely release pollen into the air

Stage 5



Note: Pollen flowers collected at stage 5 provide the best results. Stage 4 collection may readily abort if extraction conditions are not closely adhered to.

Appendix 2a

A development rating system for *Pinus banksiana* female flowers (megastrobili)

Stage

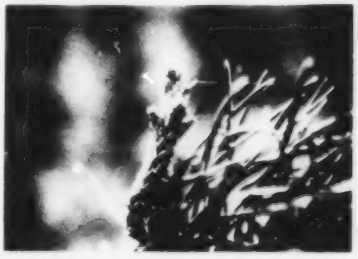
0 - buds noticeable but still very tight against the leader or new shoot

1 - tube-shaped buds are enlarged and easily recognizable (arrow)
- bud scales tight

Stage 1

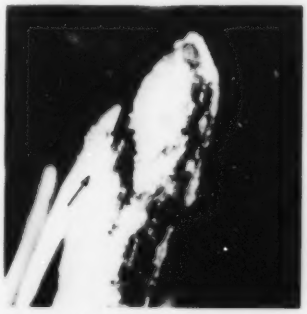
2 'juvenile' stage:
- bud scales opening at the tip (arrows)
- the cone flower is barely visible

Stage 2



3 'intermediate' stage:
- bud scales receding
- cone flower is exposed and the cone scales are beginning to open

Stage 3



4 'advanced' stage:
- cone scales approaching right angle position relative to the cone axis and beginning to swell

5 'receptive' stage:
- cone scales at right angles to the cone axis
- cone scales swollen but the space between the rows of scales is still evident

Stage 5

Bagging is done during stage 2. Early bagging will contribute to the integrity of the controlled cross as well as allow one to concentrate on pollen collection. Early bagging, however, may promote female flower development. Early collection and forced extraction of pollen can be done successfully in pines, so problems in synchronization should not be a major concern.

Pollination should be conducted when the female flowers are at late stage 4 to stage 5. For security, two pollen applications 1 to 2 days apart should be made during this receptive period.

Appendix 2b

A development rating system for *Pinus banksiana* male flowers (microstrobili)

Stage

0 - tight clusters of microstrobili very apparent and resin-covered

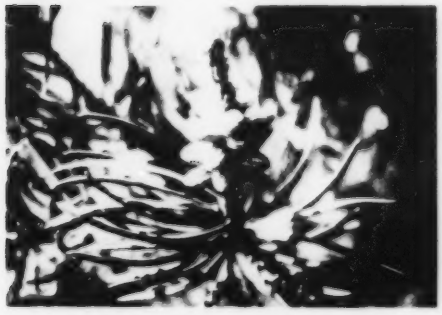
1 - clusters enlarging
- the pollen flower is hard when squeezed

Stage 1

2 'juvenile' stage:
- a clear fluid squirts out of the pollen flower when squeezed

3 'intermediate' stage:
- the clusters of microstrobili are further enlarged and a yellow fluid squirts out when squeezed

Stage 3



4 'advanced' stage:
- microstrobili flexible and spongy
- when squeezed, the thick yellow paste that is emitted almost dries when rolled between the fingers

Stage 4

5 'mature' stage:
- the cluster of microstrobili are swollen in appearance
- when squeezed they are dry and feel spongy
- the microstrobili when flicked with one's finger will freely release pollen into the air

Stage 5

Pollen clusters can be collected with good results at stage 4.

Appendix 3a

A development rating system for *Larix* spp. female flowers (megastrobili)

Stage

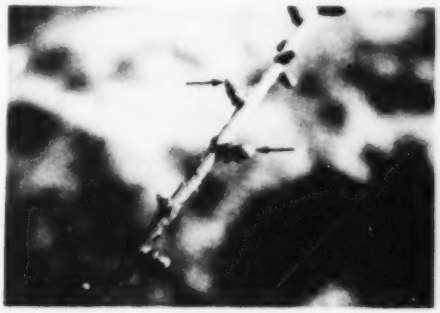
0 - pronounced, elongated bullet-shaped buds (arrows) is tightly covered by scales

1 - buds enlarged
- bud scales opening at the tip, barely exposing the cone flower

Stages 0-1

2 'juvenile' stage:
- flowers partially emerged (arrows)
- cone scales still tight

Stage 2



3 'intermediate' stage:
- cone flower has mostly emerged
- cone scales beginning to open (arrow)

Stage 3

4 'advanced' stage:
- cone flower has completely emerged
- cone scales approaching a position at right angles to the cone axis

5 'receptive' stage:
- cone scales spread and fully open (arrow)

Stage 5

Bagging should be done at stages 1 - 2. As the flowers emerge they are fragile and can easily break off if accidently hit. Pollination should be conducted when the female flowers are at late stage 4 to stage 5. For security, two pollen applications 1 to 2 days apart should be made during this period.

Early bagging will contribute to the integrity of the controlled cross, especially given the rapidity of pollen development and release in the larches. It should be noted, however, that early bagging may promote flower development. Because early collection in *Larix* and forced extraction of pollen requires detailed attention (see Copis 1990 a,b), close observations must be made to both male and female flower development to achieve synchronization.

Note: The cone flower of *Larix laricina* is much smaller and not as impressive in appearance as that in *L. decidua* and *L. leptolepis*.

Appendix 3b

A development rating system for *Larix* spp. male flowers (microstrobili)

Stage:

0 - dome-shaped buds tightly covered by bud scales (arrow)

1 - bud scales receding

Stages 0-1

2 'juvenile' stage:
- bud scales have retracted
- clear fluid squirts from the pollen flower when squeezed

3 'intermediate' stage:
- appearance more robust
- a yellow fluid squirts out when squeezed

Stage 3

4 'advanced' stage:
- pollen flower is swollen
- when squeezed it does not squirt fluid but, rather, emits a thick yellow paste that almost dries to a powder when rolled between the fingers

5 'mature' stage:
- microstrobilus beginning to 'burst' (arrow)
- when squeezed the pollen flower is dry and feels spongy
- when flicked with one's finger, the flower will freely release pollen into the air

Stage 5

Development of pollen flowers from stages 3-5 is very rapid, especially during extended warm, dry periods. Collections should be made at the onset of stage 5 for best extraction results; however, extensive monitoring of the flowers is essential so that this stage is not missed. Stage 4 collections may readily abort if precautions (such as placing cut branches in water) are not used.

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